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NASA TECH BRIEF

Goddard Space Flight Center

Improved Design of Electrophoretic Equipment for Rapid Sickle-Cell-Anemia Screening

The problem:

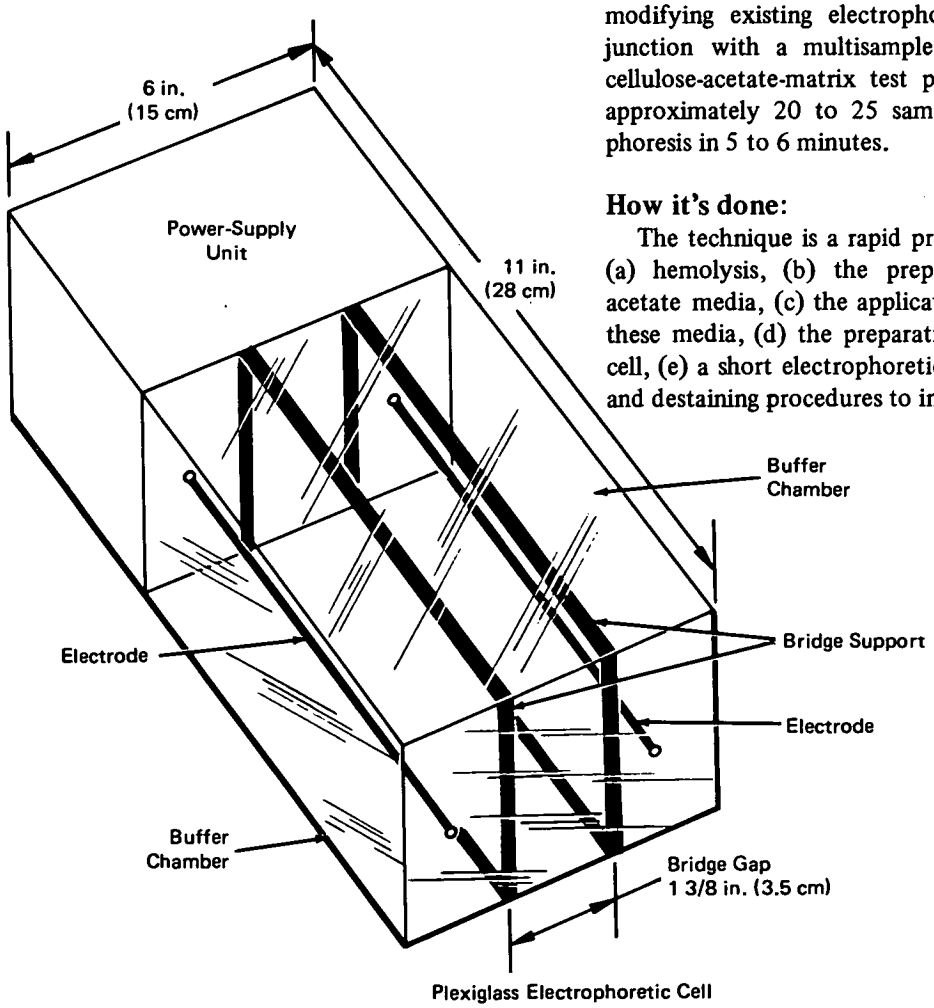
Electrophoresis has played a major role in the discovery and the characterization of the different types of hemoglobins. Included among these is hemoglobin S, the hemoglobin responsible for the sickling of red cells. The disease follows a Mendelian pattern, which could have a catastrophic effect on community health. It is therefore desirable to perform continual mass screening for both the trait and the disease. Present electrophoretic methods, however, are too slow for effective mass screening.

The solution:

Effective mass screening may be accomplished by modifying existing electrophoretic equipment in conjunction with a multisample applicator used with a cellulose-acetate-matrix test paper. Using this method, approximately 20 to 25 samples can undergo electrophoresis in 5 to 6 minutes.

How it's done:

The technique is a rapid progression of steps through (a) hemolysis, (b) the preparation of the cellulose-acetate media, (c) the application of the hemolysate to these media, (d) the preparation of the electrophoretic cell, (e) a short electrophoretic run, and (f) the staining and destaining procedures to indicate the test results.



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The first step in the analysis is the preparation of the hemolysate. Whole blood is drawn from subject's finger and is collected in two 75- by 1.3-mm heparinized microhematocrit tubes. The tubes then are sealed with a hematocrit sealing clay and are centrifuged for 15 seconds in microhematocrit centrifuge. Following centrifugation, both ampules are scored and broken in the clay area. Plasma in the segments, containing plasma and packed red blood cells, is discarded by either tilting the tubes or drawing the plasma with a syringe. The packed red blood cells are mixed for approximately 15 seconds in 2 cm^3 of a 2-percent aqueous detergent solution to release the hemoglobin, making the samples ready for the test.

A simplified diagram of the electrophoretic cell is shown in the figure. The cell electrodes are made of 60 percent platinum and 40 percent rhodium and are connected to a 500-volt dc power source. The left and right chambers of the electrophoretic cell are separated by a distance of 3.5 cm and filled with a barbital buffer solution to a depth just sufficient to cover the electrode wires. The buffer solution has a pH factor of 8.8 and an ionic strength of 0.06, diluted with distilled water to 1 liter. The supporting media used in the tests are cellulose-acetate membranes, 50- by 100-mm strips. These strips are floated in the buffer solution until saturated. The strips then are blotted between two sheets of ordinary blotting paper and these moist strips then are ready to accept the samples.

The samples are applied to the moist strips by using an applicator designed specifically for multisample application. As many as 10 hemolysate samples can be applied simultaneously to one strip. The cellulose-acetate strips with hemolysate samples then are placed on the supports of the cell, and electrophoresis is carried out at 500 volts for 5 minutes. Following electrophoresis, the strips are stained for 3 minutes in a 0.5-percent solution of Ponceau S in 7.5 percent trichloroacetic acid. Destaining is accomplished in three rinses of 2 percent acetic acid. Then the strips are air dried on sheets of blotter paper and are examined.

Note:

Requests for further information may be directed to:

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Patent status:

NASA has decided not to apply for a patent.

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